IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Walter Callen et al. Art Unit : 1652
Serial No. : 10/081,872 Examiner : Rebecca E. Prouty, Ph.D.
Filed : February 21, 2002
Title : ENZYMES HAVING AMYLASE ACTIVITY AND METHODS OF USE THEREOF

Director of the US Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

2. I declare that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art, e.g., screening enzymes, and nucleic acids encoding enzymes, for various amylase activities, e.g., alpha amylase activity, was very high. Using the teaching of the specification, one skilled in the art could have selected routine methods known in the art at the time of the invention to express variants of nucleic acids encoding the exemplary amylase of the invention and screen them for expression of polypeptides having various amylase activities. One skilled in the art could have used routine protocols known in the art at the time of the invention, including those described in the instant specification, to screen for nucleic acids encoding polypeptides having a percent sequence identity to SEQ ID NO:125,

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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Jeanne Amour
Signature

Jeanne Amour
Typed or Printed Name of Person Signing Certificate

or active fragments thereof, for various amylase activities. At the time of the invention it was routine to screen for multiple substitutions or multiple modifications of an enzyme-encoding sequence and predictably achieve positive results. While the numbers of samples needed to be screened may have been high, the screening procedures were routine and successful results predictable (i.e., it was predictable to find nucleic acids encoding amylases having various activities). Furthermore, it would not have required any knowledge or guidance as to which are the specific structural elements, e.g., amino acid residues, that correlate with amylase activity to create variants of the exemplary nucleic acid and test them for the expression of polypeptides or peptides having amylase activity. Accordingly, it would not have taken undue experimentation to make and use the claimed invention, including identification of a genus of nucleic acids encoding amylases active under various conditions.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date:_____ _____

Jay Short

CURRICULUM VITAE

NAME: Jay M. Short, Ph.D.

EDUCATION:

1981 - 1985	Ph.D., Biochemistry Case Western Reserve University, Cleveland, Ohio
1980 - 1981	Graduate Study, Macromolecular Science Case Western Reserve University, Cleveland, Ohio
1976 - 1980	B.A. with Honors, Chemistry Taylor University, Upland, Indiana

RESEARCH & PROFESSIONAL EXPERIENCE:

1999 - present	**CEO, President, Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1998 - present	**President, Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1997 - 1998	**Executive Vice President, Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1994 - 1997	**Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1990 - 1994	**President** Stratacyte, Inc. La Jolla, California
1992 - 1994	**Vice President** R&D (Research) and Operations Stratagene Cloning Systems La Jolla, California
1989 - 1992	**Vice President** R&D (Research) and Biological Operations Stratagene Cloning Systems
1988 - 1989	**Senior Staff Scientist** Research and Development Stratagene Cloning Systems

1985 - 1988	**Staff Scientist** Research and Development Stratagene Cloning Systems
1981 - 1985	**Ph.D. Research** Case Western Reserve University Dr. Richard W. Hanson's Laboratory, Identification and characterization of the promoter for P-enolpyruvate carboxykinase. First identification of a cAMP regulatory domain.
1980 - 1981	**Graduate Student Research** Case Western Reserve University Dr. Bruce Roe's Laboratory, Analysis of the cellulase activity of *Trichoderma viride*.

TEACHING EXPERIENCE:

Thesis Advisor (1988-1993), University of Uppsala, Sweden, Ph.D. for Michelle Alting-Mees
Lecturer (1992), Committee for Advanced Scientific Education, Center for Drug Evaluation and Research, FDA.
Faculty (1989), Transgenic Mouse Model and Its Application in Assessing *In Vivo* Mutagenesis, Genetic Toxicology Workshop (3rd Annual). Microbiological Associates Inc. Bethesda, MD.
Faculty (1987), DNA Cloning and Expression. Physiology Society Workshop. San Diego, CA.
Teaching Assist., (1981-1985). Molecular and Cellular Biology. Case Western Reserve University.
Teaching Assist., (1981). Physiological Chemistry. Kent State Univ., Kent, OH.
Teaching Assist., (1978-1980). Quantitative Analysis. Taylor University.

AWARDS, PROFESSIONAL MEMBERSHIPS, ACCOMPLISHMENTS, AND ACTIVITIES:

Visiting Scientist, International Centre of Insect Physiology and Ecology (ICIPE), Kenya (2002-2004)
Science & Technology Committee, *BIOCOM San Diego*
Advisory Board, IngleWood Ventures
Finalists for UCSD Connect's Most Innovative New Product Award in the Biotechnology R&D Category
Advisory Board, *Chemical & Engineering News*
Board of Advisors and Founding Member, Division of Biological Sciences, *UCSD*
Board Director, *BIOCOM San Diego*
Chairman of the Board, Innovase
Board Director, Zymetrics
Board Director, Innovase
Director at Large, *YPO (Young Presidents' Organization) San Diego.*
2001 T-Sector Life Science Innovator Award.
2001 Deloitte and Touche's Orange County / San Diego 2001 Technology "Fast 50".
San Diego Entrepreneur of the Year 2001.
YPO (Young Presidents' Organization) San Diego.
YPO (Young Presidents' Organization) International.
Finalist for San Diego Entrepreneur of the Year in 2000.
Largest Biotechnology IPO raising over $200MM.
Founding management member of Diversa Corporation.
Panel for Chemical Science & Technology for NIST, appointed by the National Research Council (1997-2000).
Chairman (1993), Discussion Group, Society of Toxicology Conference.
U.S. Committee Member for Evaluation of Biotechnology Research in Spain.
Editor, Mutation Research.

UCSD Connect Program (1991) 1st Place Award for Innovation and Entrepreneurship in Biotechnology (over 50 competing biotech companies).
UCSD Connect Program (1990) 1st Place Award for Innovation and Entrepreneurship in Biotechnology.
Consultant for European Economic Community on Transgenic Toxicology Testing (91-94).
The New York Academy of Sciences.
Reviewer for *Proceedings of the National Academy of Sciences, Genetic Analysis Techniques, Analytical Biochemistry, & Nucleic Acids Research.*
American Association for the Advancement of Science.
American Chemical Society.
American Society of Biochemistry and Molecular Biology.
American Society of Microbiology.
Environmental Mutagenesis Society.
Society for Industrial Microbiology
Society of Toxicology.
Japanese Environmental Mutagen Society.
Who's Who Registry of Business Leaders (1994-1995)
American Men and Women of Science (1995)
NIEHS Peer Review Committee.
SBIR Study Section.
SBIR Annual Report (1993) Program Success Profile (Top 8 of 800 Companies).
Stratagene (1990) Innovation Award - Lambda ZAP® vector.
Stratagene (1990) Service Award
Stratagene (1991) Innovation Award - Big Blue® Transgenic Testing System.
Stratagene (1992) Most Innovative Award - Managers/Supervisors.
Institutional Animal Care and Use Committee (IACUC), Chairman and Institutional Official.
Award from the University of Victoria for Contributions to the Development of Short-term Transgenic Mutation Assays.
Nominated as Council Member for the U.S. Environmental Mutagen Society.
Board Director, *Stressgen (TSE), Victoria, BC, Canada*
Board Director & Treasurer, *Stressgen Therapeutics, Victoria, BC, Canada*
Board Director & Secretary, *Stressgen Therapeutics, Victoria, BC, Canada*
Board Director, *Diversa, La Jolla, CA*
Board Director, *Invitrogen, Carlsbad, CA*
Consultant, *Stratagene Cloning Systems, La Jolla, CA*
Consultant, *Micro Product Systems, Lynn, IN*
Reviewer for U.S. Congressional Office of Technology Assessment (OTA) on *The Human Genome Project and Patenting DNA Sequences.*

MEDIA:

ABC Discovery News, ABC San Diego Channel 10, BBC Radio, Bioinformed Newsletter, Biotechnology Newsletter, BioVentures View, Business Daily, Business Week, CEO Cast, Chemical Engineering, Chemical Week, Chemistry & Industry (UK), CNBC, CNN Science & Technology, dBusiness.com, Discovery Magazine, Forbes.com, Good Morning America, Horizon Air Magazine, Idea TV, Inside Business Radio Show, JAG Financial News, Los Angeles Times, NBC San Diego Channel 7/39, National Radio Report, New York Times, Pirateinvestor.com, R&D Magazine, RTL German Television, Reuters, San Diego Business Transcript, San Diego Channel KUSI, San Diego Channel 10, San Diego Magazine, San Diego Union Tribune, Scientist, Time Magazine, Stewards' Watch, The Discovery Channel, The Motley Fool, Time Magazine, USA Today, Wall Street Journal, Wall Street Transcript, Washington Post

PATENTS:

DNA Cloning Vectors with *in vivo* Excisable Plasmids (1987).
Mutagenesis Testing Using Transgenic Animals Carrying Marker Genes (1987).
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test

DNA Sequences (1987).
Dietary and Hormonal Regulation of Expression of Exogenous Genes in Transgenic Animals Under Control of the Promoter of the Gene Phosphoenolpyruvate Carboxykinase (1988).
A Transgenic Mouse for Measurement and Characterization of Mutation Induction *In Vivo* (1989).
Rapid Screening Mutagenesis and Teratogenesis Assay (1989).
A Combinatorial Approach to Regenerating the Immunoglobulin Repertoire in Prokaryotic Cells (1990).
Transgenic Animal Models for *In Vivo* Mutagenesis Testing (1990).
Polycos Vectors (1991).
A Lambda Packaging Extract Lacking β-Galactosidase Activity (1991).
A System for Regulation of Eukaryotic Genes (1991).
Methods for Phenotype Creation from Multiple Gene Populations (1991).
Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
Selectable System Patent (1992).
Polycos Mutagenesis Systems (1993).
Use of Trans-acting Proteins for the Development of an *In Situ* Expression Screening System (1993).
Enzyme Kits and Libraries (1995).
Enzyme Activity Screening of Clones having DNA from Uncultivated Microorganisms (1995).
Enzyme Tiered (1995).
Method for Screening for Enzyme Activity (1995).
Combined Enzyme Screening/Evolution (1995).
Uncultured/Activity Screening (1995).
Directed Evolution of Thermophilic Proteins (1995).
Combinatorial Enzyme Development (Directed Mutagenesis) (1996).
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms (1996).
Production and Use of Normalized DNA Libraries (1996).
Methods of DNA Shuffling with Polynucleotides Produced by Blocking or Interrupting a Synthesis or Amplification Process (1996).
Method of Screening for Enzyme Activity (Biopanning) (1996).
Directed Evolution of Thermophilic Enzymes (1996).
Environmental Biopanning (1996).
Combinatorial Enzyme Development (1996).
Enzyme Activity Screening of Clones Having DNA from Uncultivated Miroorganisms (1996).
Normalized Samples/Libraries (1996).
Reassembled Pools of Mutagenized DNA & Procedure (1996).
Fluorescent-based Single Screening for Enzymes (1996).
High Throughput Screening for Novel Enzymes (1997).
Nucleotide Sequence of the *Aquifex aeolicus* Genome, Fragments Thereof, and Uses Thereof (1997).
Screening for Novel Bioactivities (1997).
Screening for Novel Compounds which Regulate Biological Interactions (1997).
Method for Screening Enzyme Activity (1997).
High Throughput Screening for Novel Enzymes (1997).
"Discovery" (whole process, including uncultivated, normalized, biopanning, screening, evolving, (etc.) (1997).
Production of Enzymes Having Desired Activities By Mutagenesis (1999).
Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms (1999).
Method of DNA Reassembly by Interrupting Sythesis (1999).
Production and Use of Normalized DNA Libraries (1999).
Enzyme Kits and Libraries (1999).
Screening for Novel Bioactivities (2000).
Method for Screening for Enzyme Activity (2000).
Screening for Novel Bioactivities (2000).
Production and Use of Normalized DNA Libraries (2000).
Method of Screening for Enzyme Activity (2000).
Screening Methods for Enzymes and Enzyme Kits (2001).
Saturation Mutagenesis in Directed Evolution (2001).
High Throughput Screening for Novel Enzymes (2001).

Recombinant Bacterial Phytases and Uses Thereof (2001).
Methods Useful for Nucleic Acid Sequencing Using Modified Nucleotides Comprising Phenylboronic Acid (2001).
End Selection in Directed Evolution (2001)
Gene Expression Library Produced From DNA From Uncultivated Microorganisms and Method for Making the Same (2001)
Directed Evolution of Thermophilic Enzymes (2002)
Method for Screening for Enzyme Activity (2002)
Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
End Selection In Directed Evolution (2002)
Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
Screening for Novel Bioactivities(2002)
Method of DNA Shuffling with Polynucleotides Produced or Blocking or Interrupting Synthesis or Amplification Process (2002)
Production and Use of Normalized DNA Libraries (2002)
Sequence Based Screening (2002)
Non-Stochastic Generation of Genetic Vaccines (2002)
Over 100 Additional Pending Patent Applications Worldwide.

GRANTS AND CONTRACTS:

*Phase I Small Business Contract #N43-Am-62282. 1985 - 1986. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
*Phase II Small Business Contract #N43-Am-62282. 1988 - 1990. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
*Phase I Small Business Grant 2R43ES04484-02. 1986 - 1987. P.I.
Identification of Genetic Lesions Leading to Mutations.
*Phase II Small Business Grant 2R43ES04484-02. 1989 - 1992. P.I.
Identification of Genetic Lesions Leading to Mutations.
*1R01-ES04728-01A1. 1989 - 1992. (NIEHS) P.I.
Animal Model for Identification of Genetic Lesions.
*Phase I Small Business Grant #R43GM42291-01. 1989. P.I.
Switch Mechanism for Gene Expression in Transgenics.
*RFP NIH-ES-88-11. 1989-1994. (NIEHS) Co-I.
Development of Mutagenesis Assays Using Transgenic Mice.
*Phase II Small Business Grant #2R44GM42291-02. 1990-1992. (DRG/NIH) P.I.
Switch Mechanism for Gene Expression in Transgenics.
*Phase I Small Business Grant #1R43GM46585-01. 1991. (DRG/NIH) P.I.
Generation of a Peptide Screening System Through the Development of Combinatorial-splicing "Polycos" Vectors.
*Phase I Small Business Grant #1R43CA57066-01. 1992. (NCI) P.I.
Transgenic Rats: A Short-term Mutagenicity Assay for Multi-species Testing of Suspected Human Carcinogens.
*Phase I Small Business Grant #1R43GM48300-01. 1992. (DRG/NIH) P.I.
Analysis of the Immunoglobulin Hypermutator Mechanism.
*Phase I Small Business Grant #1R43ES06146-01. 1992. (NIEHS) P.I.
Selectable "Polycos" Shuttle Vectors for In Vivo Mutagenicity Testing.
*Phase II Small Business Grant #2R44GM46585-02. 1992-1994. (NIGMS) P.I.
Peptide Screening Utilizing Combinatorial Polycos Vector.
*Phase I Small Business Grant #1R43RR08667-01. 1992-1993. (DRG/NIH) Co-I.
A One-step PCR Cloning System Based on FLP Recombination.
*Phase II Small Business Grant #2R44CA57066-02. 1993-1995. (NCI) P.I.
Transgenic Rats:Transgenic Rat Model for Mutagenicity Testing.
*Phase I Small Business Grant. 1993-1994. (NIH) Co-I.
Transgenic Fish Model for Mutagenicity Testing.
*Phase II Small Business Grant (1994-1996). (NIH) P.I.
"Polycos" Shuttle Vectors for Mutagenicity testing.
*Phase I Small Business Grant. 1994. (NIH) Co-I.
Vector System for Studying Protein-Protein Interactions.

*CRADA with LLNL. 1994. (NIH) Co-I.
Mouse and Rat Painting Probes.
*CRADA with FDA. 1994. (NIH) Co-I.
Tamoxifen Testing in F-344 Rats.
*CRADA with NASA. 1994. (NIH) Co-I.
Radiation Damage in the Microgravity Environment.

ABSTRACTS AND INVITED LECTURES:

Over 200 Abstracts and Invited Lectures.

PUBLICATIONS:

1. Yoo-Warren, H., Monahan, J.E., Short, J.M., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H.M., Samols, D., and Hanson, R.W. (1983) Isolation and Characterization of the Gene Coding for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) from the Rat. *Proc. Natl. Acad. Sci. U.S.A.,* 80:3656-3660.

2. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1984) Identification of cAMP Regulatory Region in the Gene for Rat Cytosolic Phosphoenolpyruvate Carboxykinase (GTP): Use of Chimeric Genes Transfected into Hepatoma Cells. *J. Biol. Chem.,* 259:12161-12169.

3. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1985) A Region of the Gene for Rat Cytosolic P-enolpyruvate Carboxykinase Confers cAMP Responsiveness to the HSV-thymidine Kinase Gene. In: *Membrane Receptors and Cellular Recognition,* (M. Czech and C.R., Kahn, eds.), Alan Liss Inc., New York, pp 339-346.

4. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. I. Multiple Hormone Regulatory Elements and the Effects of Enhancers. *J. Biol. Chem.,* 261:9714-9720.

5. Short, J.M., Wynshaw-Boris, A., Short, H.P., and Hanson, R. W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. II. Identification of cAMP and Glucocorticoid Regulatory Domains. *J. Biol. Chem.,* 261:9721-9726.

6. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) The Determination of Sequence Requirements for Hormonal Regulation of Gene Expression. *Biotechniques,* 4:104-119.

7. Burns, D.M., Bhandari, G., Short, J.M., Sanders, P.G., Wilson, R.H., and Miller, R.E. (1986) Selection of a Rat Glutamine Synthetase cDNA Clone. *Biochemical and Biophysical Research Communications,* 134:146-151.

8. Hod., Y. Cook, J.S., Weldon, S.L., Short, J.M., Wynshaw-Boris, A., and Hanson, R.W. (1986) Differential Expression of the Genes for the Mitochondrial and Cytosolic Forms of P-enolpyruvate Carboxykinase Gene. In: *Metabolic Regulation: Application of Recombinant DNA Techniques,* (A.G., Goodridge and R.W. Hanson eds.), Annals of the New York Academy of Sciences, New York, Vol. 278, pp. 31-45.

9. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1987) *cis* - acting Regulatory Elements in Hormonally Responsive Genes. In: *Progress in Nucleic Acid Research and Molecular Biology* (W.E. Cohn and K. Moldave eds.), Academic Press, Inc., Orlando, Florida, 34:59-87.

10. Bullock, W., Fernandez, J.M., and Short, J.M. (1987) XL1-Blue: A High Efficiency Plasmid Transforming *rec*A *E.coli* Strain With β-Galactosidase Selection. *Biotechniques,* 5:60-64.

11. Short, J.M., Fernandez, J.F., Sorge, J.A., and Huse, W. (1988) Lambda ZAP®: A Bacteriophage Lambda Expression Vector With *In Vivo* Excision Properties. *Nucleic Acids Res.,* 16:7583-7600.

12. Short, J.M. (1988) Book Review: Vectors - A Survey of Molecular Cloning Vectors and Their Uses. Raymond L. Rodriques and David T. Denhardt, eds, Butterworths, Stoneham, MA. *Genomics*, 2:270-271.

13. Short, J.M., and Pollard, A. (1988) Gigapack XL: Size Selective Packaging Extract. *Strategies in Mol. Biol.*, 1:5-7.

14. Kretz, P.L., and Short, J.M. (1989) Gigapack II: A Restriction Deficient (*mcrA-, B-, hsd-, mrr-*) Lambda Packaging Extract. *Strategies in Mol. Biol.*, 2(2):25-26.

15. Kretz, P.L., Reid, C.H., Greener, A., and Short, J.M. (1989) Effect of Lambda Packaging Extract Mcr Restriction Activity on DNA Cloning. *Nucleic Acids Res.* 17:5409.

16. Sastry, L., Alting-Mees, M., Huse, W.D., Short, J.M., Sorge, J.A., Hay, B.N., Janda, K.D., Benkovic, S.J., and Lerner, R.A. (1989) Cloning of the Immunological Repetoire in *E. coli* for Generation of Monoclonal Catalytic Antibodies I. Construction of a V_H Specific cDNA Library. *Proc. Natl. Acad. Sci. U.S.A.*, 86:5728-5732.

17. Short, J.M. (1989) The Use of Lambda Phage Shuttle Vectors in Transgenic Mice for Development of a Short Term Mutagenicity Assay. In: *Fifth International Conference on Environmental Mutagens*, Alan Liss, Inc., New York,Part A:335-367. Article and Lecture.

18. Alting-Mees, M., and Short, J.M. (1989) pBluescript II: Gene Mapping Vectors. *Nucleic Acids Res.*, 17:9494.

19. Shopes, B., Alting-Mees, M., Amber, J.R., Ardourel, D., Callahan, M., Detrick, J., Hay, B.N., Hogrefe, H.H., Greener, A., Gross, E.A., Kubitz, M.M., Mullinax, R.L., Wilson, C., Short, J.M., and Sorge, J.A. (1990) Bacteriophage Immuno-expression Libraries: An Emerging Technology for the Identification and Production of Monoclonal Antibodies. *Antibody Engineering, New Tech. & Application Implications.* pp. 98-101.

20. Alting-Mees, M., Amberg, J., Ardourel, D., Elgin, E., Greener, A., Gross, E.A., Kubitz, M., Mullinax, R.L., Short, J.M., and Sorge, J.A. (1990) Monoclonal Antibody Expression Libraries:A Rapid Alternative to Hybridomas. *Strategies in Mol. Biol.*, 3:1-9.

21. Kohler, S., Provost, S., Dycaico, M., Sorge, J., and Short, J.M. (1990) Development of a Short-term, *In Vivo* Mutagenesis Assay: The Effects of Methylation on the Recovery of a Lambda Phage Shuttle Vector from Transgenic Mice. *Nucleic Acids Res.*, 18:3007-3013.

22. Kohler, S., Provost, G.S., Kretz, P.L., Fieck, A., and Short, J.M. (1990) An *In Vivo* Assay Using Transgenic Mice to Analyze Spontaneous and Induced Mutations at the Nucleic Acid Level. *Stratagies in Mol. Biol.*, 3:19-21.

23. Kretz, P., Kohler, S., and Short, J.M. (1990) The Effect of *E. coli* Minute 98 Locus on DNA Containing Eukaryotic Modifications. *Strategies in Mol. Biol.*, 3:21-22.

24. Mullinax, R.L., Gross, E.A., Amberg, J., Hogrefe, H., Kubitz, M., Greener, A., Alting-Mees, M., Ardourel, D., Hay, B.N., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Identification of Human Antibody Fragment Clones Specific for Tetanus Toxin in a Bacteriophage Lambda Immuno-Expression Library. *Proc. Natl. Acad. Sci. U.S.A.*, 87:8095-8099.

25. Cline,J., Lundberg, K., Nielson, K., Sorge, A., Short, J.M., and Mathur, E.J. (1990) StrataClean Resin: Non-Toxic Protein Extraction. *Strategies in Mol. Biol.*, 4(4):49-51.

26. Mullinax, R.L., Gross, E.A., Amber, J.R., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Human Antibody Clones Isolated From a Bacteriophage Lambda Immunoexpression Library. *Strategies in Mol. Biol.*, 4(4):51-52.

27. Provost, G.S., Kohler, S.W., Fieck, A., Kretz, P.L., Molina, T., and Short, J.M. (1990) Short-term Germ Line and Somatic Cell Mutagenesis Testing With *LacI* Lambda Phage Shuttle Vectors in Transgenic Mice. *Strategies in Mol. Biol.*, 4(4):55-56.

28. Kohler, S.W., Provost, G.S., Kretz, P.L., Fieck, A., Sorge, J.A., and Short, J.M. (1990) The Use of Transgenics Mice for Short Term, *In Vivo* Mutagenicity Testing. *Genetic Analysis Techniques,* 7(8):212-218.

29. Shopes, B., Mullinax, R.L., Amber, J.R., Gross, E.A., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., and Sorge, J.A. (1990) ImmunoZAPÆ Bacteriophage Libraries: A New Technology for the Identification and Expression of Monoclonal Antibodies. *Biotech USA Conference Proceedings,* pp.332-341.

30. Raleigh, E.A., Benner, J., Bloom, F., Braymer, H.D., DeCruz, E., Dharmalingam, K., Heitman, J., Noyer-Weidner, M., Piekarowicz, A., Kretz, P.L., Short, J.M., and Woodcock, D. (1991) Nomenclature Relating to Restriction of Modified DNA in *Escherichia coli. Journal of Bacteriology,* 173(8):2707-2709.

31. Kretz, P., Kohler, S., and Short, J.M. (1991) Identification and Characterization of a Gene Responsible for Inhibiting Propagation of Methylated DNA Sequences in *mcrA, mcrB1 E. coli* Strains. *Journal of Bacteriology,* 173:4707-4716.

32. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, B., Sorge, J, A., Putman, D., and Short, J.M. (1991) Spectra of Spontaneous and Induced Mutations Using a Lambda ZAP® *LacI* Shuttle Vector in Transgenic Mice. *Proc. Natl. Acad. Sci. U.S.A.,* 88(18):7958-7962.

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35. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, W.O., Putman, D.L., Sorge, J.A., and Short, J.M. (1992) Analysis of Spontaneous and Induced Mutations in Transgenic Mice Using a Lambda ZAP®/*LacI* Shuttle Vector. *Environmental and Molecular Mutagenesis,* 18:316-321.

36. Fieck, A., Wyborski, D., and Short, J.M. (1992) Modifications of the *E. coli Lac* Repressor for Expression in Eukaryotic Cells: Effects of Nuclear Signal Sequences on Protein Activity and Nuclear Accumulation. *Nucleic Acids Research,* 20:1785-1791.

37. Hay, B., and Short, J.M. (1992) ExAssist™ Helper Phage and SOLR™ Cells for Lambda ZAP® II Excisions. *Strategies in Mol. Biol.,* 5:16-18.

38. Short, J.M. (1992) Tissue Specific Mutagenesis in Transgenic Mice. *The Toxicology Forum, 1992 Annual Winter Meeting,* pp.79-109.

39. Alting-Mees, M.A., Sorge, J.A., and Short, J.M. (1992) pBluescript II: Multifunctional Cloning and Mapping Vectors. *Methods in Enzymology,* 216:483-495.

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Yellowstone Center for Resources (2002). Kerry M. Murphy (kerry_murphy@nps.gov), P.O. Box 168, Yellowstone National Park, WY 82190.

Exhibit A

1: Nucleic Acids Res. 1992 Mar 11;20(5):1083-5.

Related Articles, Li

Stable propagation of cosmid sized human DNA inserts in an F factor based vector.

Kim UJ, Shizuya H, de Jong PJ, Birren B, Simon MI.

Division of Biology, California Institute of Technology, Pasadena 91125.

Instability of complex mammalian genomic DNA inserts is commonplace in cosmid libraries constructed in conventional multicopy vectors. To develop a means to construct stable libraries, we have developed a low copy number cosmid vector based on the E. coli F factor replicon (Fosmid). We have teste relative stability of human DNA inserts in Fosmids and in two conventional multicopy vectors (Lawrist 16 and Supercos) by comparing the frequency of changes in restriction patterns of the inserts after propagating randomly picke human genomic clones based on these vectors. We found that the clones base on Fosmid vector undergo detectable changes at a greatly reduced frequency. We also observed that sequences that undergo drastic rearrangements and deletions during propagation in a conventional vector were stably propagatec when recloned as Fosmids. The results indicate that Fosmid system may be useful for constructing stable libraries from complex genomes.

PMID: 1549470 [PubMed - indexed for MEDLINE]

1: Genet Anal. 1995 Oct;12(2):81-4.

Construction and utility of a human chromosome 22-specific Fosmid library.

Kim UJ, Shizuya H, Sainz J, Garnes J, Pulst SM, de Jong P, Simon MI.

Division of Biology, California Institute of Technology, Pasadena 91125, US

We have previously demonstrated the capability of the Fosmid vector based on Escherichia coli F-factor replicon to stably propagate cosmid-sized human genomic DNA fragments. Using the Fosmid vector, we have constructed and arrayed a 10 x human chromosome 22-specific library, partly by picking human positive clones from a total Fosmid library constructed using DNA from human-hamster hybrid cell line containing human chromosome 22, and partly by using flow-sorted chromosomal DNA. The clones and physical contig map of the clones in the library will serve as a valuable resource for detailed analysis of the chromosome by providing reliable materials for high resolution mapping and sequencing. In order to efficiently build physical maps for the chromosomal regions of interest spanning several hundred kilobases to a megabase, it is necessary to rapidly identify subsets of the Fosmid clones from the library that cover such regions. In this report, we describe a method of using random amplification products derived from YAC clones to rapidly identify a subset of Fosmid clones that cover a specific genomic subregion.

PMID: 8574898 [PubMed - indexed for MEDLINE]

☐ 1: Mol Gen Genet. 2000 May;263(4):648-54. Related Articles, Li

Transformation of Arabidopsis with a Brassica SLG/SRK regio and ARC1 gene is not sufficient to transfer the self-incompatibility phenotype.

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Department of Molecular Biology and Genetics, University of Guelph, Ontar Canada.

Self-incompatibility (SI) promotes outbreeding in flowering plants, and in Brassica SI is genetically controlled by the S locus. Self-incompatible Brassi and self-fertile Arabidopsis belong to the same crucifer family. In addition, a comparative analysis reveals a high degree of microsynteny between the B. campestris S locus and its homologous region in Arabidopsis--with the notab exception that the Brassica SI genes, SLG and SRK, are missing. Brassica ARC1 encodes a component of the SRK signal transduction pathway leading self-pollen rejection, and no closely related ARC1 homolog has been identifi in Arabidopsis. The purpose of the research reported here was to introduce Brassica SI components into Arabidopsis in an attempt to compensate for the missing genes and to investigate whether the SI phenotype can be transferred Inserts of approximately 40 kb from the fosmid clones F20 and F22, which span the B. napus W1 SLG-SRK region, were cloned into the plant transformation vector pBIBAC2. Transgenic plants were generated that expressed the Brassica SI genes in the flower buds. In addition, the endogenous, SLG-like, gene AtS1 was not co-suppressed by the Brassica SL transgene. No SI phenotype was observed among the T1 BIBAC2-F20 and BIBAC2-F22 transgenic plants. When the ARC1 gene was transformed into BIBAC2-F20 or BIBAC2-F22 plants, the resulting BIBAC2-F20-ARC1 and BIBAC2-F22-ARC1 plants still set seeds normally, and no rejection respons was observed when self-incompatible B. napus W1 pollen was placed on BIBAC2-F20-ARC1 or BIBAC2-F22-ARC1 Arabidopsis stigmas. Taken together, our results suggest that complementing Arabidopsis genome with Brassica SLG, SRK and ARC1 genes is unlikely to be sufficient to transfer tl SI phenotype.

PMID: 10852487 [PubMed - indexed for MEDLINE]

Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A → B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.